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	L3	L2 and (\$toxin or toxi\$).clm.	25
	L2	L1 and (pseudomonas or pseudo-monas or pe)	196
	L1	(fitzgerald\$.in. or Fitzgerald\$.in.)	4864

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Brief Summary Text:

[0013] In another aspect, the invention provides a chimeric protein comprising: (a) a non-toxic <u>Pseudomonas</u> exotoxin A sequence comprising domain Ia, domain II, and domain III; and (b) a Type IV pilin loop sequence, wherein the Type IV pilin loop sequence is located between domain II and domain III of the non-toxic <u>Pseudomonas</u> exotoxin A sequence.

Brief Summary Text:

Charles

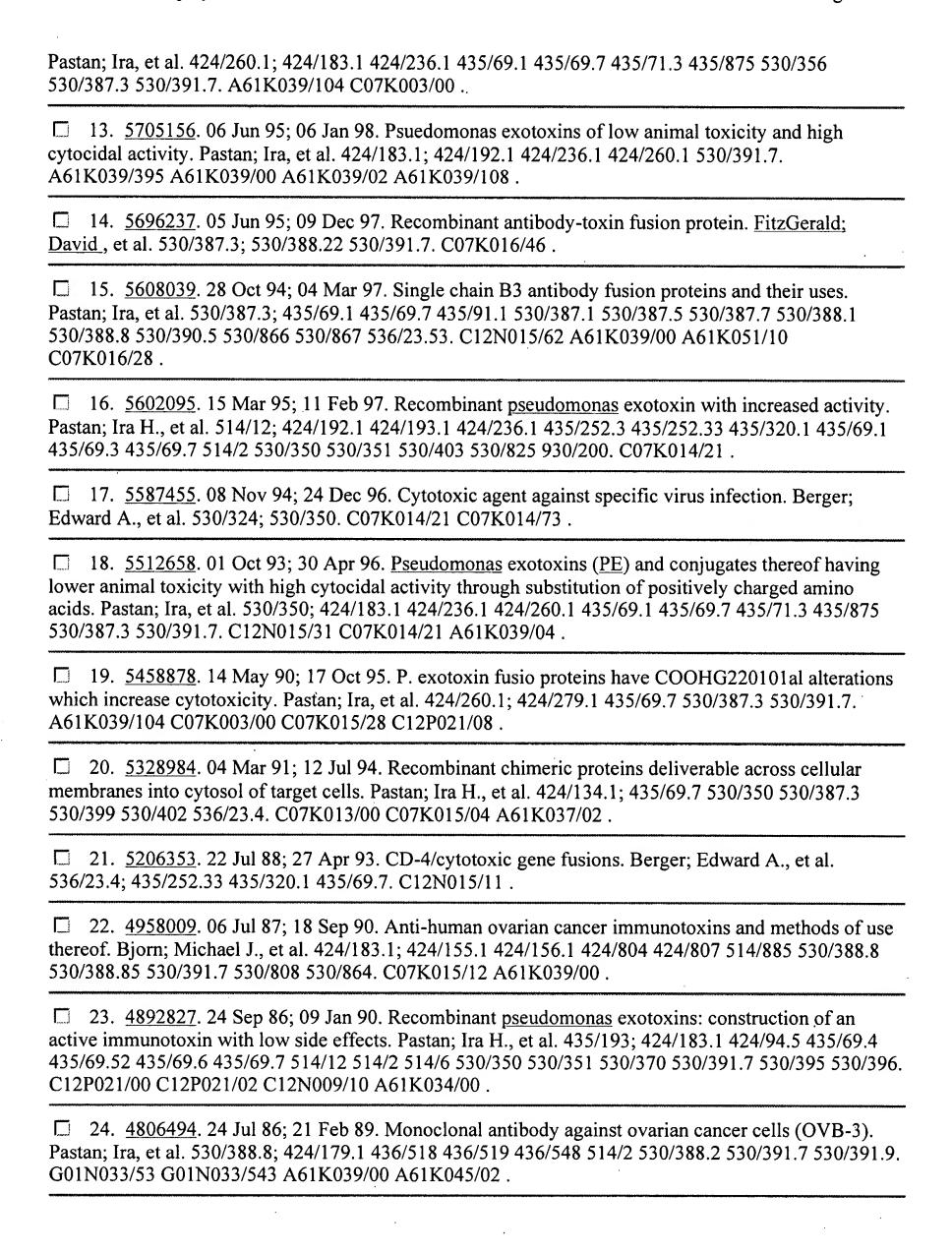
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Description of Disclosure:

[0029] "Pseudomonas exotoxin A" or "PE" is secreted by P. aeruginosa as a 67 kDa protein composed of three prominent globular domains (Ia, II, and III) and one small subdomain (Ib) connecting domains II and III. (Allured et. al., Proc. Natl. Acad. Sci. 83:1320-1324 (1986).) Domain Ia of PE located at the N-terminus and mediates cell binding. In nature, domain Ia binds to the low density lipoprotein receptor-related protein ("LRP"), also known as the .alpha.2-macroglobulin receptor (".alpha.2-MR"). (Kounnas et al., J. Biol. Chem. 267:12420-23 (1992).) It spans amino acids 1-252. Domain II mediates translocation to the cytosol. It spans amino acids 253-364. Domain lb has no known function. It spans amino acids 365-399. Domain III is responsible for cytotoxicity and includes an endoplasmic reticulum retention sequence. It mediates ADP ribosylation of elongation factor 2 ("EF2"), which inactivates protein synthesis. It spans amino acids 400-613. The native Pseudomonas aeruginosa exotoxin A nucleic acid sequence and the amino acid sequence are shown as SEQ ID NO:1 and SEQ ID NO:2, respectively. SEQ ID NOS: 1 and 2 are the mature form of exotoxin A, wherein the signal sequence has been cleaved off. As a virulence factor, PE can kill PMNs, macrophages and other elements of the immune system (Pollack et al., Infect. Immuno. 19(3):1092-6 (1978)).

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- my seperior 200 **Search Results** - Record(s) 1 through 25 of 25 returned. 1. 20070003578. 15 May 06. 04 Jan 07. Chimeric protein comprising non-toxic pseudomonas exotoxin and type IV pilin sequences. Fitzgerald; David J. . 424/236.1; 424/260.1 A61K39/108 20060101 2. 20050079171. 09 Sep 03. 14 Apr 05. Pseudomonas exotoxin A-like chimeric immunogens for eliciting a secretory IgA-mediated immune response. FitzGerald, David J., et al. 424/133.1; 61K039/395. 10/659,036 ch 3. 20040071731. 21 May 03. 15 Apr 04. Chimeric protein comprising non-toxic pseudomonas exotoxin a and type iv pilin sequences. Fitzgerald, David J., 424/190.1; 435/252.3 435/320.1 435/69.3 530/395 536/23.7 A61K039/02 C07H021/04 C07K014/195 C12P021/02 C12N001/21. 4. 20030054012. 12 May 00. 20 Mar 03. PSEUDOMONAS EXOTOXIN A-LIKE CHIMERIC IMMUNOGENS FOR ELICITING A SECRETORY IGA-MEDIATED IMMUNE RESPONSE. FITZGERALD, DAVID J., et al. 424/190.1; 435/5 A61K039/02 C12Q001/70 5. 6881718. 17 Apr 02; 19 Apr 05. Disulfide conjugated cell toxins and methods of making and using them. FitzGerald; David J., et al. 514/2; 514/14 514/15 530/300 530/344. C07K001/36 C07K002/00 A61K038/00. 6. 6426075. 30 Jul 99; 30 Jul 02. Protease-activatable pseudomonas exotoxin A-like proproteins. <u>Fitzgerald</u>; David J., et al. 424/260.1; 424/183.1 424/184.1 424/193.1 424/193.1 424/236.1 424/261.1 435/69.1 435/69.7 435/71.1 435/71.3 530/356 530/387.3 530/391.7. **X**61K039/108 A61K039/00 C12P021/04 C12P021/06 C12N015/09. omanas 7. 6423513. 10 Jan 00; 23 Jul 02. Polynucleotides encoding protease-activatable pseudomonas exotoxin a-like proproteins. Fitzgerald; David J., et al. 435/71.3; 424/183.1 424/184.1 424/192.1 424/193.1 424/236.1 424/260.1 435/252.33 435/320.1 435/69.1 435/69.3 435/69.7 435/71.1 530/351 530/356 530/387.3 530/391.7. C12P021/04 C12P021/06 C12N015/09 A61K039/00. 8. 6099842. 03 Dec 90; 08 Aug 00. Recombinant immunotoxin composed of a single chain antibody reacting with the human transferrin receptor and diptheria toxin. Pastan; Ira, et al. 424/183.1; 424/178.1 530/391.7. A61K039/40 A61K039/42 A61K039/44 A61K039/395 . 9. <u>5863745</u>. 05 Jun 95; 26 Jan 99. Recombinant antibody-toxin fusion protein. Fitzgerald; David J. , et al. 435/7.21; 424/134.1 424/183.1 530/387.3 530/388.22 530/391.7. G01N033/563 C07K016/46. 10. <u>5854044</u>. 05 Jun 95; 29 Dec 98. Recombinant <u>pseudomonas</u> exotoxin with increased activity. Pastan; Ira H., et al. 435/194; 530/324 530/350 530/351 530/387.3 530/387.7 530/399. C07K019/00 C12N009/12. 11. <u>5821238</u>. 05 Jun 95; 13 Oct 98. Recombinant <u>pseudomonas</u> exotoxin with increased activity. Pastan; Ira H., et al. 424/134.1; 424/179.1 424/183.1 424/832 435/69.7 514/12 530/350 530/387.1 530/387.3 530/387.7 530/391.7 530/825. A61K039/104 A61K038/43 C07K014/21 C12P021/02. 12. <u>5705163</u>. 05 Jun 95; 06 Jan 98. Target-specific, cytotoxic, recombinant <u>pseudomonas</u> exotoxin.



□ 25. 4545985. 26 Jan 84; 08 Oct 85. Pseudomonas exotoxin conjugate immunotoxins. Pastan; Ira, et al. 424/180.1; 424/179.1 514/2 514/6 530/388.22 530/388.23 530/391.9 530/404 530/414 530/806 530/807 530/825 530/826 530/828. A61K039/00 A61K039/02 A61K037/00 A23J001/06.

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-continued

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Lys Asp Glu Leu

(2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Trp Glu Gln Leu Glu

What is claimed is:

1. A protease-activatable Pseudomonas exotoxin A-like ("PE-like") proprotein comprising:

(a) a cell recognition domain that binds to an exterior 25 SKGKGTSSQYSNTE (SEQ ID NO:13). surface of a targeted cell;

- (b) a modified PE translocation domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 280 to 344 of SEQ ID NO:2 and which effects translocation to a cell cytosol upon proteolytic cleavage, wherein the translocation domain comprises a cysteine-cysteine loop that comprises a protease activatable sequence cleavable by a protease and wherein the protease activatable sequence is refractory to cleavage by furin when incubated with furin at a 1:10 enzyme:substrate molar ratio at 25° C. for 16 hour;
- (c) a cytotoxicity domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 400 to 613 of SEQ ID NO:2, the cytotoxicity domain having ADP-ribosylating activity; and
- (d) an endoplasmic reticulum ("ER") retention sequence.
 2. The PE-like proprotein of claim 1 wherein the modified
 PE translocation domain has a PE domain II sequence
 (amino acids 253-364 of SEQ ID NO:1) modified with
 amino acids substitutions introducing the protease activat-

able sequence so as to cause cleavage by the protease

between amino acids 279 and 280.

3. The PE-like proprotein of claim 1 wherein the protease activatable sequence is cleavable by a protease secreted by a cancer cell.

4. The PE-like proprotein of claim 1 wherein the cell recognition domain comprises an antibody that specifically binds to a cancer cell surface marker.

5. The PE-like proprotein of claim 2 wherein the protease activatable sequence is cleavable by prostate specific antigen ("PSA").

6. The PE-like proprotein of claim 2 wherein the protease activatable sequence is cleavable by urokinase.

7. The PE-like proprotein of claim 2 wherein the protease activatable sequence is cleavable by neutral endoprotease, stromelysin, collagenase, cathepsin B, or cathepsin D.

8. The PE-like proprotein of claim 2 further comprising a PE Ib domain, and wherein said PE Ib domain, the cytotoxicity domain, and the ER retention sequence together have the sequence of domains Ib and III of native PE.

9. The PE-like proprotein of claim 3 wherein the cell 65 recognition domain is coupled to the modified translocation domain through a peptide bond.

10. The PE-like proprotein of claim 5 wherein the protease activatable sequence is SKGSFSIQYTYHV (SEQ ID NO:11), HLGGSQQLLHNKQ (SEQ ID NO:12), or SKGKGTSSQYSNTE (SEQ ID NO:13).

11. The PE-like proprotein of claim 6 wherein the protease activatable sequence is DRVYIHPF (SEQ ID NO:3), VVCGERGFFYTP (SEQ ID NO:4), FFYTPKA (SEQ ID NO:5), KRRPVKVYP (SEQ ID NO:6), 30 PVGKKRRPVKVY (SEQ ID NO:7), KPVGKKRRPVKV (SEQ ID NO:8), GKPVGKKRRPVK (SEQ ID NO:9), or TFAGNAVRSVGQ (SEQ ID NO:10).

12. The PE-like proprotein of claim 8 wherein the cell recognition domain is an antibody coupled to the modified translocation domain through a peptide bond and wherein the antibody specifically binds a cancer cell surface marker.

13. A composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a protease-specific Pseudomonas exotoxin A-like ("PE-like") proprotein comprising:

- (a) a cell recognition domain that binds to an exterior surface of a targeted cell;
- (b) a modified PE translocation domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 280 to 344 of SEQ ID NO:2 and which effects translocation to a cell cytosol upon proteolytic cleavage, wherein the translocation domain comprises a cysteine-cysteine loop that comprises a protease activatable sequence cleavable by a protease and wherein the protease activatable sequence is substantially un-activatable by fibrin when incubated with furin at a 1:10 enzyme:substrate molar ratio at 25° C. for 16 hours;
- (c) a cytotoxicity domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 400 to 613 of SEQ WD NO:2, the cytotoxicity domain having ADP-ribosylating activity; and

(d) an endoplasmic reticulum ("ER") retention sequence.

14. The composition of claim 13, further comprising a PE

15-like domain, wherein:

- (a) the cell recognition domain is an antibody coupled to the modified PE translocation domain through a peptide bond and wherein the antibody specifically binds a cancer cell surface marker;
- (b) the modified PE translocation domain has a PE domain II sequence (amino acids 253-364 of SEQ ID

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- NO:1) modified with amino acids substitutions introducing the protease activatable sequence so as to cause cleavage by the protease between amino acids 279 and 280; and
- (c) the PE Ib-like domain, the cytotoxicity domain and the ER retention sequence together have the sequence of domains Ib and III of native PE.
- 15. The composition of claim 14 wherein the protease activatable sequence is cleavable by prostate specific antigen or urokinase.
- 16. A method for killing a cancer cell comprising contacting the cell with a protease-specific Pseudomonas exotoxin A-like ("PE-like") proprotein comprising:
 - (a) a cell recognition domain that binds to an exterior surface of a targeted cell;
 - (b) a modified PE translocation domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 280 to 344 of SEQ ID NO:2 and which effects translocation to a cell cytosol upon proteolytic cleavage, wherein the translocation domain

- comprises a cysteine-cysteine loop that comprises a protease activatable sequence cleavable by a protease and wherein the protease activatable sequence cysteine-cysteine loop is substantially un-activatable by furin when incubated with furin at a 1:10 enzyme:substrate molar ratio at 25° C. for 16 hours;
- (c) a cytotoxicity domain comprising an amino acid sequence with 80% or greater sequence identity to amino acids 400 to 613 of SEQ ID NO:2, the cytotoxicity domain having ADP-ribosylating activity; and
- (d) an endoplasmic reticulum ("ER") retention sequence.
- 17. The method of claim 16 wherein the cancer cell is a prostate cancer cell.
 - 18. The method of claim 16 wherein the cancer cell is a colon cancer cell.
 - 19. The method of claim 16 used in the treatment of a subject suffering from cancer.

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What is claimed is:

1. A method of making a cell toxin comprising

reacting a polypeptide comprising a *Pseudomonas* exotoxin translocation domain linked to a *Pseudomonas* exotoxin ADP-ribosylatin domain, wherein the *Pseudomonas* exotoxin translocation domain comprises at least one reactive sulfhydryl group, with a substance P peptide comprising one additional cysteine residue at its amino terminal end, wherein the cysteine sulfhydryl group is disulfide linked to a di-thiobis (2-nitro)-benzoic acid group, such that after the reaction a disulfide bond is formed between the exotoxin polypeptide and the substance P and a thionitrobenzoate group is released, and

purifying the substance P-Pseudomonas exotoxin disulfide linked conjugate from the released thionitrobenzoate group such that the purified conjugate is substantially free of thionitrobenzoate groups.

- 2. The method of claim 1, wherein the *Pseudomonas* exotoxin translocation domain sulfhydryl group located within ten amino acid residues of the translocation domain 45 amino terminus.
- 3. The method of claim 1, wherein the *Pseudomonas* exotoxin translocation domain sulfhydryl group located at the translocation domain amino terminus.
- 4. The method of claim 1, wherein the *Pseudomonas* 50 exotoxin translocation domain sulfhydryl group is a cysteine residue.
- 5. The method of claim 1, wherein the *Pseudomonas* exotoxin translocation domain is covalently linked to the *Pseudomonas* exotoxin ADP-ribosylation domain.
- 6. The method of claim 5, wherein the covalent linkage between the Pseudomonas exotoxin translocation domain and the *Pseudomonas* exotin ADP-ribosylation domain is a peptide bond.
- 7. The method of claim 1, wherein the *Pseudomonas* 60 exotoxin translocation domain comprises an amino acid sequence as set forth in SEQ ID NO:1 and the Pseudomonas exotoxin ADP-ribosylation domain comprises an amino acid sequence as set forth in SEQ ID NO:2.
- 8. A pharmaceutical composition for the ablation of NK1 65 receptor expressing cells comprising a cell toxin and a pharmaceutically acceptable excipient,

wherein the cell toxin is a substance P-Pseudomonas exotoxin disulfide linked conjugate made by a process comprising the following steps:

reacting a polypeptide comprising a Pseudomonas exotoxin translocation domain linked to a Pseudomonas exotoxin ADP-ribosylation domain, wherein the Pseudomonas exotoxin translocation domain comprises at least one reactive sulfhydryl group, with a substance P peptide comprising one additional cysteine residue at its amino terminal end, wherein the cysteine sulfhydryl group is disulfide linked to a di-thiobis (2-nitro)-benzoic acid group, such that after the reaction a disulfide bond is formed between the exotoxin polypeptide and the substance P and a thionitrobenzoate group is released, and

purifying the substance P-Pseudomonas exotoxin disulfide linked conjugate from the released thionitrobenzoate group such that the purified conjugate is substantially free of thionitrobenzoate groups.

9. The pharmaceutical composition of claim 8, wherein the *Pseudomonas* exotoxin translocation domain sulfhydryl group located within ten amino acid residues of the translocation domain amino terminus.

10. The pharmaceutical composition of claim 8, wherein the *Pseudomonas* exotoxin translocation domain sulfhydryl group located at the translocation domain amino terminus.

11. The pharmaceutical composition of claim 8, wherein the *Pseudomonas* exotoxin translocation domain sulfhydryl group is a cysteine residue.

- 12. The pharmaceutical composition of claim 8, wherein the *Pseudomonas* exotoxin translocation domain is covalently linked to the *Pseudomonas* exotoxin ADP-ribosylation domain.
- 13. The pharamceutical composition of claim 12, wherein the covalent linkage between the *Pseudomonas* exotoxin translocation domain and the *Pseudomonas* exotoxin ADP-ribosylation domain is a peptide bond.
- 14. The pharmaceutical composition of claim 8, wherein the *Pseudomonas* exotoxin translocation domain comprises an amino acid sequence as set forth in SEQ ID NO:1 and the *Pseudomonas* exotoxin ADP-ribosylation domain comprises an amino acid sequence as set forth in SEQ ID NO:2.
- 15. The pharmaceutical composition of claim 8, wherein the cell toxin and pharamceutically acceptable excipient are

suitable for administration intrathecally, subdurally or directly into the brain parenchyma.

16. A method for ablating an NK1 receptor expressing cell in a patient comprising administering to said patient a cell toxin in a pharmaceutically acceptable excipient in an 5 amount sufficient to ablate an NK1 receptor expressing cell,

wherein the cell toxin is a substance P-Pseudomonas exotoxin disulfide linked conjugate made by a process comprising the following steps:

reacting a polypeptide comprising a Pseudomonas exotoxin translocation domain linked to a Pseudomonas exotoxin ADP-ribosylation domain, wherein the Pseudomonas exotoxin translocation domain comprises at least one reactive sulfhydryl group, with a substance P peptide comprising one additional cysteine residue at its amino terminal end, wherein the cysteine sulfhydryl group is disulfide linked to a di-thiobis (2-nitro)-benzoic acid group, such that after the reaction a disulfide bond is formed between the exotoxin polypeptide and the substance P and a thionitrobenzoate group is released, and

purifying the substance P-Pseudomonas exotoxin disulfide linked conjugate from the released thionitrobenzoate group such that the purified conjugate is substantially free of thionitrobenzoate groups.

17. The method of claim 16, wherein the ablated NK1 receptor expressing cell is a dorsal horn cell, a stratum cell or a brain parenchyma cell.

18. A method of treating chronic pain without significantly affecting basal nociceptive responses comprising

administering to a subject in need thereof a cell toxin in a pharmaceutically acceptable excipient in an amount sufficient to treat chronic pain without significantly affecting basal nociceptive responses,

wherein the cell toxin is a substance P-Pseudomonas exotoxin disulfide linked conjugate made by a process comprising the following steps:

reacting a polypeptide comprising a Pseudomonas exotoxin translocation domain linked to a Pseudomonas exotoxin ADP-ribosylation domain, wherein the Pseudomonas exotoxin translocation domain comprises at least one reactive sulfhydryl group, with a substance P peptide comprising one additional cysteine residue at its amino terminal end, wherein the cysteine sulfhydryl group is disulfide linked to a di-thiobis (2-nitro)-benzoic acid group, such that after the reaction a disulfide bond is formed between the exotoxin polypeptide and the substance P and a thionitrobenzoate group is released, and

purifying the substance P-Pseudomonas exotoxin disulfide linked conjugate from the released thionitrobenzoate group such that the purified conjugate is substantially free of thionitrobenzoate groups.

19. The method of claim 18, wherein cell toxin is administered to epineurium cells, perineurium cells, nerve ganglia, nerve sheathers, nerve linings, meninges, pia mater cells, arachnoid membrane cells, dura membrane cells, cells lining a joint or brain or spinal cord parenchymal cells.

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